Serum interferon-γ is associated with longitudinal decline in lung function among asthmatic patients: the Normative Aging Study

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Background: Cytokines are important mediators of the asthmatic response. A retrospective pilot study showed that serum levels of interleukin (IL)-5 and interferon (IFN)-γ were related to lung function decline among asthmatic patients over the preceding 3 years. To confirm these findings, we tested the hypothesis that serum cytokines are associated with longitudinal ... ng function decline.

Methods: We conducted a prospective, longitudinal study of 25 asthmatic and 50 nonasthmatic men (median age, 63 years; range, 45 to 80 years) participating in the Normative Aging Study. All study subjects completed two consecutive triennial examinations, including spirometry, methacholine challenge testing, allergy skin testing, and phlebotomy. Serum levels were measured for IL-4, IL-5, IL-6, IL-8, IL-10, and IFN-γ.

Results: Among asthmatic patients, a higher initial serum level of IFN-γ was associated with a greater rate of decline of forced expiratory volume in 1 second (FEV_1; $\beta = -67$ mL/year per log increase in serum IFN-γ, $P = 0.04$) and, to a lesser extent, of FEV_1/forced vital capacity ratio ($\beta = -0.91\%$/year per log increase in serum IFN-γ, $P = 0.07$) after adjusting for age, smoking status, and baseline level of lung function. Serum IL-5 level was associated with a rate of decline in FEV_1 of borderline significance ($\beta = -61$ mL/year per log increase in serum IL-5, $P = 0.08$) among asthmatic patients. These relationships were not observed among nonasthmatic patients.

Conclusions: Serum levels of IFN-γ are associated with subsequent rate of change in lung function among asthmatic patients in this cohort of middle-aged and older men, and may be useful as biologic markers of risk for accelerated lung function decline in population studies.

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INTRODUCTION

Lung function, particularly expiratory flow rates, declines with age beginning in the third decade of life. Cigarette smoking and some environmental and occupational exposures can significantly increase the rate of longitudinal lung function decline and hasten the development of chronic obstructive pulmonary disease (COPD). Asthma has also been shown to be associated with accelerated lung function decline.[1–5] The mechanisms responsible for accelerated rates of decline are poorly understood. Prominent hypotheses have included disordered trypsin-antitrypsin balance, host susceptibility factors such as airways hyperresponsiveness or atopy, chronic airway inflammation, and airway wall remodeling. A major limitation to epidemiologic investigations of these hypotheses has been a lack of effective, noninvasive biologic markers (biomarkers) to identify subjects at risk for accelerated lung function decline.

Many of the proposed mechanisms underlying chronologic lung function decline involve an inflammation of the bronchioles. Studies demonstrating associations between eosinophilia and longitudinal lung function decline suggest that inflammatory events in the lungs may be reflected in the peripheral blood.[1] Cytokines play a critical regulatory role in many immunologic responses in the lung, including airway inflammation associated with asthma.[6,7] These small glycoproteins are produced locally at sites of inflammation, immune activation, or other physiologic activity but may diffuse

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into surrounding tissues and ultimately into the peripheral circulation. Recent improvements in detection techniques have shown that most cytokines are present at low levels in the peripheral blood of healthy, as well as diseased, subjects. Because cytokines are intimately related to the inflammatory response, we reasoned that peripheral blood cytokine levels might be useful biomarkers in longitudinal studies of lung function decline. A retrospective pilot study revealed that serum levels of interleukin (IL)-5 and interferon (IFN)-γ measured at the end of followup was related to lung function decline among asthmatic patients over the preceding 3 years.[8] To confirm the utility of serum cytokines as biomarkers of lung function decline, we carried out a prospective nested case-control study of asthmatic patients and nonasthmatic patients participating in the Normative Aging Study (NAS). Based on the results of the retrospective pilot study, we were primarily interested in the effects of IL-5 and IFN-γ on longitudinal lung function decline, but also investigated the effects of other serum cytokines: IL-4, IL-6, IL-8, and IL-10.

METHODS

Study Population

The study sample for this analysis was drawn from the NAS, a longitudinal study of health and aging established in 1961 by the Veterans Administration at the Boston Veterans Administration outpatient clinic.[9] Subjects have returned for periodic evaluations every 3 to 5 years.

All study subjects completed standardized questionnaires based on the American Thoracic Society questionnaire (ATS-DLD-1978).[10] Current smokers were defined as men who were smoking one cigarette per day within 1 month before the examination. Former smokers were defined as men who had previously smoked at least one cigarette per day but who had ceased smoking at least 1 month before the examination. Never smokers were defined as men who had never smoked cigarettes or who had smoked less than a total of 20 packs during their lifetime. Subjects who reported that they had been given the diagnosis of asthma by a physician were considered to have doctor-diagnosed asthma.

All study subjects had completed two sequential triennial screening examinations between 1984 and 1994. The time of the first examination during this period was designated as time 1 or the baseline examination, and the time of second triennial examination was designated as time 2. Cases and controls were defined on the basis of their asthma status at the initial visit of the two examinations analyzed. Cases were defined as subjects who had self-reported wheezing apart from colds on most days and either doctor-diagnosed asthma or a measured provacative dose causing a 20% or greater fall in forced expiratory volume in 1 second (PD_{20} FEV_1) <8 μmol methacholine. Controls were defined as subjects who reported that they had never been told by a physician that they had asthma, had never had wheezing apart from colds on most days, and consistently had PD_{20} FEV_1 >50 μmol methacholine. Nonasthmatic patients for the present study were selected by stratified random sampling across quintiles of FEV_1 decline to ensure that a range of rates of decline in FEV_1 was represented. The study population consisted of 25 asthmatic patients and 50 nonasthmatic patients. The study was approved by the Human Studies Subcommittee, Department of Veterans Affairs Outpatient Clinic; written informed consent was obtained from all subjects.

Spirometry and Methacholine Challenge Protocol

Spirometry and methacholine challenge were performed as previously described.[11,12] The methacholine challenge protocol was adapted from that described by Chatham et al.[13] Lung function decline was defined as lung function (in milliliters for FEV_1 and forced vital capacity [FVC], and the ratio of FEV_1/FVC $\times$ 100 for FEV_1/FVC %) at the end of the followup period (time 2) minus lung function at the beginning of the followup period (time 1). This difference was divided by the number of years of followup to obtain decline in lung function per year: milliliters (mL)/year for FEV_1 and FVC, and percent (%)/year for FEV_1/FVC %.

Skin Testing

After completion of the methacholine challenge test, skin testing was performed as previously described,[11] by the prick method of Pepys.[14] Subjects were tested in double-blind fashion with four common aeroallergen preparations preserved in glycerin (ragweed, 1:20; mixed trees, 1:20; mixed grasses, 1:20; and house dust, 1:10) along with a glycerin control. Wheal reactions were measured at 20 minutes as the sum of the largest wheal diameter plus the perpendicular diameter divided by two. A positive skin test was defined as subjects having a wheal diameter of $\geq$2 mm to any of the above allergens, after subtraction of the glycerin control.

Chemiluminescent Enzyme-Linked Immunoadsorbent Assay (CL-ELISA)

Blood was collected by routine phlebotomy in evacuated tubes, allowed to clot, and the serum separated and frozen until testing. The concentrations of IL-4, IL-5, IL-6, IL-8, IL-10, and IFN-γ in serum were determined by CL-ELISA as previously described.[15] Briefly, purified cytokine capture (coating) monoclonal antibodies (MAB) [anti-IL-4, anti-IL-5, and anti-IL-10 MAB (Pharmingen, San Diego, CA); anti-IL-6, anti-IFN-γ MAB, and anti-granulocyte-macrophage colony-stimulating factor MAB (Endogen, Boston, MA); anti-IL-8 (R & D Systems, Minneapolis, MN)] for each cytokine were diluted in coating buffer (0.1 M $NaHCO_3$, pH 8.2). One hundred microliters of the appropriate MAB were added to each well of a 96-well flat-bottom opaque microtiter immunoassay plate (Microlite 1, Dynatech Laboratories, Chantilly, VA). After overnight incubation, each well was blocked with 200 μL/well of 2.5% (weight/volume) bovine serum albumin (BSA)/phosphate-buffered saline (PBS)-Tween. One hundred microliters per well of serum diluted 1:4 with PBS-Tween or recombinant human cytokine standard [IL-4, IL-5, and IL-10 (Pharmingen); IL-6 (Genzyme); IL-8 (R & D Systems); and IFN-γ (Endogen)] diluted with 1.0% BSA/

PBS-Tween were added in triplicate wells. After incubation, each plate was washed with PBS-Tween, and biotinylated anti-cytokine detecting MAB in 0.5% BSA/PBS-Tween was added to each well [anti-IL-4, IL-5, and IL-10 MAB (Pharmingen); anti-IL-6, [anti-IFN-γ MAB] (Endogen); anti-IL-8 (R & D Systems)]. ExtrAvidin Alkaline phosphatase (Sigma), diluted 1:10,000 with 0.5% BSA/PBS-Tween, was then added at 100 μL/well, followed by 100 μL/well Lumi-Phos 530 (Lumigen, Southfield, MI). Light emission from each plate was measured with a microtiter plate luminometer (ML 1000; Dynatech Laboratories). A standard curve for each plate was computed, and results for the test samples were calculated from the standard curve by extrapolation with BioCalc Data Analysis Software (BioCalc J. V., Herndon, VA). The detection limits were also determined at that time: 1.25 pg/mL for IL-5 and IFN-γ; 2.5 pg/mL for IL-4, IL-8, and granulocyte-macrophage colony-stimulating factor; 2 pg/mL for IL-6; and 0.6 pg/mL for IL-10.

These CL-ELISAs have been validated for accuracy and sensitivity with recombinant human cytokines, including World Health Organization standard recombinant cytokines where available. World Health Organization standards include: recombinant IFN-γ (catalog no Gg23-901-530) from the National Institute of Allergy and Infectious Disease, National Institutes of Helath, Bethesda, MD; recombinant human IL-4 (88/656) and recombinant human IL-5 (90/586) from the National Institute for Biologic Standards and Control, Hertfordshire, England. The assays were tested extensively using media and human serum samples spiked with mixtures of known quantities of recombinant cytokines. Each assay is highly specific for the cytokine of interest, with no detectable cross-reactivity with other cytokines. These studies confirmed the quality assurance data provided by the manufacturers of the MAB pairs.

Data Analysis

Because serum cytokine levels are approximately log normally distributed in healthy populations,[16] cytokine levels were logarithmically transformed (log10) for all analyses. Mean values for continuous variables, both between groups and across the 3-year time interval were compared using Student *t* test. Two outcomes were defined for this analysis. For the cross-sectional analyses, the outcome was baseline lung function (ie, FEV_1, FVC, and FEV_1/FVC at time 1). For the longitudinal change analyses, the outcome was lung function decline (as defined in the section on spirometry above). Multiple linear regression analysis was used to control for the influence of selected covariates on baseline lung function and lung function decline. All descriptive and analytical statistics were performed using the SAS statistical package (SAS Institute, Cary, NC).

RESULTS

Characteristics of the Study Population

The median age of the study participants was 63 years (range = 45 to 80 years). As a group, asthmatic patients had a significantly lower mean percent predicted FEV_1, FVC, and FEV_1/FVC ratio than did nonasthmatic patients at the beginning of the 3-year followup period (time 1; Table 1). Asthmatic patients also had significantly higher eosinophil counts and percentage of eosinophils, than did nonasthmatic patients. There were no significant differences in age or leukocyte counts. A greater proportion of asthmatic patients had a positive skin test to the panel of allergens than nonasthmatic patients. Similar differences were noted at the end of the followup period (time 2; data not shown). There were no significant differences in serum cytokine levels between asthmatic patients and nonasthmatic patients at either time point.

Relationship of Serum Cytokine Levels to Cross-Sectional Pulmonary Function

In cross-sectional analyses, IL-5 serum levels were inversely related to FEV_1 and FVC at time 1 in linear regression models controlling for age and cigarette smoking (Table 2). Stratified analysis demonstrated a similar magnitude of effect among both asthmatic patients and nonasthmatic patients. Similar (but nonsignificant) relationships were observed at time 2. The concentrations of IL-4, IL-6, IL-8, IL-10, and IFN-γ were not related to the levels of FEV_1, FVC, or FEV_1/FVC ratio.

Serum Cytokines and Longitudinal Change in Pulmonary Function

The combined study population exhibited an average yearly decrease of 28 mL for FEV_1, 25 mL for FVC, and 0.17% for FEV_1/FVC ratio. There was no significant difference between longitudinal pulmonary function changes between the asthmatic patients and nonasthmatic patients, likely reflecting the stable nature of asthmatic patients in this cohort and the fact that selection of nonasthmatic patients was performed by stratified random sampling across quintiles of FEV_1 decline. However, asthmatic patients exhibited a much wider variability in their longitudinal lung function changes than the nonasthmatic patients.

To evaluate relationships between serum cytokine levels and rate of change in pulmonary function, each cytokine was incorporated as a covariate in regression models that adjusted for known determinants of lung function decline: age, smoking, and baseline lung function. In these models, IFN-γ was associated with longitudinal decline in pulmonary function among asthmatic patients (Table 3). Serum IFN-γ level was significantly associated with decline in FEV_1 and to a lesser extent with FEV_1/FVC ratio. IL-5 level was associated with a borderline significant decline in FEV_1 among asthmatic patients in the adjusted model. No significant relationships were observed for the serum levels of the other cytokines.

DISCUSSION

The purpose of this investigation was to evaluate the potential value of serum cytokines, primarily IL-5 and IFN-γ, as biomarkers to predict longitudinal changes in lung function. Preliminary data from an earlier retrospective pilot study had

Table 1. Baseline (Time 1) Characteristics among Asthmatic Subjects and Nonasthmatic Subjects

	Asthma (n = 25)	No asthma (n = 50)	*P* Value
Lung function: % predicted (mean ± SE)			
FEV_1	80 ± 3	98 ± 2	<0.0001
FVC	89 ± 3	100 ± 2	<0.01
FEV_1/FVC	89 ± 2	98 ± 1	<0.0001
Hematology (mean ± SE)			
WBC (cells/mm^3)	7104 ± 408	6416 ± 212	NS
Eosinophils (cells/mm^3)	304 ± 42	183 ± 13	0.01
% Eosinophils	4.5 ± 0.7	3.0 ± 0.2	<0.05
Basophils (cells/mm^3)	40 ± 6	37 ± 3	NS
% Basophils	0.66 ± 0.08	0.58 ± 0.04	NS
Skin test status: number of subjects (%)			
Positive*	17 (77.3%)	25 (52.1%)	<0.05
Smoking status: number of subjects (%)			
Current smoker	3 (12%)	3 (6%)	NS
Former smoker	16 (64%)	30 (60%)	NS
Never smoked	6 (24%)	17 (34%)	NS
Symptoms: number of subjects (%)			
Hay fever	9 (35%)	8 (16%)	0.05
Cough	11 (44%)	1 (2%)	0.001
Phlegm	14 (56%)	5 (10%)	0.001
Dyspnea	2 (0.8%)	0	<0.05
Wheeze	25 (100%)	2 (4%)	0.001
Log_{10} serum cytokines (mean ± SE)			
IL-4	1.34 ± 0.14	1.27 ± 0.08	NS
IL-5	0.82 ± 0.62	0.75 ± 0.06	NS
IL-8	0.67 ± 0.19	0.53 ± 0.10	NS
IL-10	2.46 ± 0.10	2.38 ± 0.08	NS
IFN-γ	0.58 ± 0.13	0.57 ± 0.07	NS

* Any wheal ≥2 mm to ragweed, grass, tree, or house dust (after subtraction of glycerin control); see Methods for details. Three cases and two controls had missing data for skin testing.

SE, standard error; FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; WBC, white blood cell count; IL, interleukin; IFN, interferon.

Table 2. Multiple Linear Regression Models Relating Level of Pulmonary Function to Serum IL-5 Level

	Model FEV_1 (L)		Model FVC (L)		Model FEV_1/FVC (%)	
	β (SE)	*P* Value	β (SE)	*P* Value	β (SE)	*P* Value
Intercept	6.1 (0.6)	0.0001	7.8 (0.7)	0.0001	83.3 (6.6)	0.0001
Age (years)	−0.04 (0.01)	0.0001	−0.05 (0.01)	0.0001	−0.05 (0.10)	0.6
Smoking						
Current	−0.87 (0.29)	0.004	−0.83 (0.02)	0.02	−7.8 (3.2)	0.02
Former	−0.21 (0.16)	0.2	−0.06 (0.19)	0.7	−4.3 (1.7)	0.01
Log_{10} serum IL-5 (pg/mL)	−0.31 (0.15)	0.04	−0.36 (0.18)	0.04	−0.9 (1.6)	0.6
Model R^2	0.3		0.3		0.1	

IL, interleukin; FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; SE, standard error.

suggested that certain cytokines (IL-5 and IFN-γ) might be useful markers of lung function decline.[8] However, in that study cytokine testing was performed at the end of the observation period (ie, time 2), and thus, a prospective, longitudinal association could only be inferred. The current prospective study design was developed to test the hypothesis that serum cytokine levels would predict *subsequent* lung function decline, adjusting for known determinants of lung function decline (age, cigarette smoking status, and initial level of lung function). The results demonstrated that serum IFN-γ levels and, to a lesser extent, serum IL-5 levels were significantly associated with decline in FEV_1 and FEV_1/FVC ratio among asthmatic patients. To our knowledge, this is the first report of a relationship between serum cytokine levels and longitudinal lung function decline in subjects with asthma.

Table 3. Relationship of Serum Cytokine Levels to Longitudinal Change in Lung Function*

	IFN-γ		IL-5	
	β	(P Value)	β	(P Value)
Asthmatic Subjects				
ΔFEV_1 (mL/yr)	−67	(0.04)	−61	(0.08)
ΔFVC (mL/yr)	−62	(0.2)	−70	(0.3)
$\Delta FEV_1/FVC$ (%/yr)	−0.9	(0.07)	−0.5	(0.3)
Nonasthmatic subjects				
ΔFEV_1 (mL/yr)	−3	(0.9)	19	(0.4)
ΔFVC (mL/yr)	−3	(0.9)	28	(0.3)
$\Delta FEV_1/FVC$ (%/yr)	−0.1	(0.8)	−0.01	(0.98)

* Regression coefficients and significance estimate (P) for change in pulmonary function (ΔFEV_1, ΔFVC, $\Delta FEV_1/FVC$) over a 3-year interval are derived from linear regression model adjusting for initial FEV_1, age, and cigarette smoking status.
IFN, interferon; IL, interleukin; FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity.

In vitro, IFN-γ is produced by TH1 lymphocytes, a designation based on murine CD4+ helper T-cell clones that can be characterized into unique functional subsets based on their pattern of cytokine production. TH1 cells also produce IL-2, whereas TH2 cells produce IL-4, IL-5, IL-6, and IL-10.[17] These patterns of cytokine production also correspond to functional specificity, with TH1 cytokines involved primarily in cell-mediated responses and inflammation, and TH2 cytokines involved in allergic reactions and antihelminthic responses. These subsets appear to cross-regulate one another, with TH1 responses inhibited by IL-4 and IL-10, and TH2 responses depressed by IFN-γ.[18,19] Alternatively, some cell populations produce cytokine profiles that have features of both TH1 and TH2, and are designated as TH0,[20,21] and produce IL-2, IL-4, IL-5, and IFN-γ.

Studies using specimens obtained by bronchial biopsy[22] and bronchoalveolar lavage (BAL)[23] have shown that the airways of asthmatic patients are characterized by cell populations with a predominant TH2 pattern of cytokine production. Thus, our finding of a significant cross-sectional inverse association between IL-5 and pulmonary function is consistent with the TH2 hypothesis of asthma pathogenesis. Whereas IFN-γ is thought to play an inhibitory role in the TH2 asthma state,[18,19,24] several studies reporting elevated levels of IFN-γ from asthmatic patients suggest that this cytokine may play a different role in this disease, at least in adults, than previously appreciated. Krug et al[25] observed an increased percentage of T cells producing IFN-γ in BAL fluid from asthmatic patients compared with that from nonasthmatic patients. A similar, but nonsignificant, elevation of IFN-γ positive T cells was observed in the peripheral blood of asthmatic patients. IFN-γ–producing T cells far outnumbered IL-4–producing T cells both in asthmatic patients and nonasthmatic patients. Others[26] have likewise found elevated IFN-γ levels in cell culture supernatants from asthmatic BAL specimens compared with those from controls. Elevated IFN-γ has also been reported in peripheral blood of asthmatic patients. Corrigan and Kay[27] found elevated levels of IFN-γ in serum of acute severe asthmatic patients. These elevated levels returned to normal after appropriate therapy. Interestingly, nonatopic asthmatic patients in that study seemed to have more systemic immune "activation," with elevated serum IFN-γ levels and soluble IL-2 receptors, than allergic asthmatic patients, suggesting a prominent role for cell mediated inflammation among nonatopic subjects. Elevated serum IFN-γ has been associated with airways hyperresponsiveness and increased circadian peak flow variation in asthmatic patients,[15] and serum IFN-γ levels were also found to be associated with the level of bronchial responsiveness to methacholine among nonasthmatic young women.[28]

Although the results from these studies and ours appear to contradict the currently accepted hypothesis of asthma pathogenesis, the studies cited above have not addressed an important issue, namely, the identity of the cells that produce IFN-γ in asthmatic patients. The recent work of Magnan et al[29] may suggest an answer. They found that IFN-γ levels in whole blood culture supernatants from atopic and nonatopic asthmatic patients were significantly elevated compared with those from either nonatopic nonasthmatic controls or atopic nonasthmatic controls, and was likely attributable to the increased proportion of IFN-γ–producing CD8+ T cells in the blood of asthmatic patients. Further, they found that this increased IFN-γ–generating capacity of CD8+ T cells was related to asthma severity, bronchial hyperresponsiveness, and blood eosinophilia. This is distinct from other atopic diseases characterized by a shift toward TH2 responses with high IL-4 production and inhibition of IFN-γ production by CD4+ T cells.[18,19,29] Further evidence comes from murine models in which IFN-γ[30] and CD8+ T cells[31] are required for the development of airway hyperresponsiveness. These results are in line with the increasingly recognized link between IFN-γ–generating CD8+ T cells and asthma.[29,32]

Asthmatic patients are known to have accelerated lung function decline compared with nonasthmatic patients,[1–5] which is thought to be secondary to the chronic airway inflammation and its contribution to airway wall remodeling.[6,33] However, we did not find a significant difference in pulmonary function declines between asthmatic patients and nonasthmatic patients in our study, despite the lower lung function values observed at Time 1 for the asthmatic patients. This fact likely reflects the relatively stable nature of asthmatic patients in our cohort. Further, we only observed subjects at two timepoints. Despite these limitations, our results indicate that among asthmatic patients in our study, there are ongoing mechanisms, which are reflected in serum levels of cytokines, that may predispose some asthmatic patients to have greater lung function declines than others. One hypothesis that may explain our result is that IFN-γ and IL-5 serum levels may reflect two elements of the airway inflammation that are believed to play a role in long-term lung function decline. IFN-γ has been associated with nonallergic, cell-mediated inflammation[19] and may also be linked with allergic

inflammation,[29,32] whereas IL-5 has been associated with eosinophilic inflammation.[24] In addition, the recent finding that transgenic mice that overexpress IFN-γ in the lung develop pathophysiologic changes of local tissue inflammation, alveolar enlargement, and increased lung volumes consistent with emphysema[34] provides further evidence for a role of IFN-γ in chronic lung disease and lung function decline. The cross-sectional relationship between serum IL-5 levels and lung function among both asthmatic patients and nonasthmatic patients suggests that this cytokine could be a useful marker for adverse pulmonary effects unrelated to airway responsiveness.

Several other limitations are apparent in this current analysis. The design and size of the present study did not allow us to address issues of airway responsiveness among nonasthmatic patients or to distinguish between atopic and nonatopic asthmatic patients. Because of the age of the subjects in this study, we can not entirely exclude the possibility that some of the cases had concomitant COPD, or would go on to develop COPD at a later date. This is not very likely, however, as majority of the subjects were either former smokers or never smokers. Because we measured serum, not BAL, cytokine levels, we can not be certain that the lung was the site of cytokine production, and we can only hypothesize that the serum cytokine levels that we have measured reflects, in part, processes that are occurring in the lung. Further, these levels reflect "basal" unstimulated levels. However, our results are consistent with studies cited above that have shown elevated levels of IFN-γ in BAL specimens and peripheral blood of asthmatic patients and suggest a role for IFN-γ in the pathogenesis of pulmonary function impairment in asthma. Although it is recognized that cytokine levels either in peripheral blood or in BAL specimens may fluctuate depending on the clinical situation (ie, whether or not a subject was having an asthma exacerbation), our subjects were clinically stable and free of overt symptoms of asthma exacerbation at the time of testing. Finally, we also did not have information on other situations which may alter cytokine levels (eg, allergen immunotherapy[35]). However, we did have information on steroid use among the cases. Only four (16%) of the cases were on steroids at the time of the study. Although INF-γ levels were lower in those who were taking steroids compared with the cases who were not on steroids (0.2 ± 0.7 vs 0.6 ± 0.7), this difference was not statistically significant ($P = 0.3$). There were no differences in IL-5 levels among cases who were on steroids compared with cases not on steroids (0.8 ± 0.8 vs 0.8 ± 0.6, $P = 0.9$). Further, removal of these four cases from the linear regression models did not appreciably change the results.

CONCLUSION

We have demonstrated that serum level of IFN-γ is a significant predictor of the rate of decline of FEV_1 among asthmatic patients in the NAS population, after adjusting for age, smoking status, and baseline level of lung function. IL-5 showed a borderline significant relationship to the rate of decline. To our knowledge, this is the first report of a relationship between serum cytokine levels and longitudinal lung function decline in asthmatic patients. As indicators of cellular inflammation, IFN-γ and IL-5 may represent useful biomarkers for identifying those asthmatic patients who are at increased risk for an accelerated, potentially irreversible, lung function decline in future population studies. In this context, these serum cytokines may also be useful as markers of therapeutic response to anti-inflammatory therapy directed at interrupting the process of inflammation and airway damage.

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